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FORMATION, ORGANIZATION, AND COMPOSITION OF THE EGG CAPSULE OF THE MARINE GASTROPOD, *ILYANASSA OBSOLETA*

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Abstract

Embryos of the marine mud snail *Ilyanassa obsoleta* undergo early development within an egg capsule. After about a week of encapsulation, embryos hatch by releasing a chemical substance that removes the plug found at the apex of a capsule. However, the mechanism of action of this hatching substance remains poorly understood. To study how the hatching substance functions, we examined the composition of the egg capsule, particularly the plug region, to determine what the "substrate" of the hatching substance might be. We have also examined the formation and organization of the egg capsule to determine the origin and identity of the regions of a capsule that the hatching substance must remove. The results show that the *Ilyanassa* egg capsule is organized into four layers, the outer three of which are composed of protein and carbohydrate. Portions of the two inner layers of the capsule wall extend into the capsule apex and form the plug, which is dissolved by the hatching substance. The isolated capsule plug region contains three major glycoproteins resolved on sodium dodecyl sulfate-polyacrylamide gels. Therefore, the hatching substance may be a protease similar in action to the enzymes released by many other embryos at hatching.

Introduction

The egg capsules of prosobranch gastropod molluscs are examples of the various types of extracellular envelopes that surround the embryos of animals. Egg capsules are produced in the maternal oviduct by a process described in detail for four species of gastropods by Fretter (1941). The process may be similar for other species as well. Eggs are released from the ovary and are fertilized in the upper regions of the oviduct. The fertilized eggs, in groups of up to several hundred, move down the oviduct to a specialized region, the albumen gland, and are embedded in the secretions of this gland. A sphincter between the albumen gland and the next region of the oviduct, the capsule gland, relaxes and the embryos are forced into the protein and mucus secretions of the capsule gland. This movement of the embryos leaves an opening in the secretions that is subsequently sealed by the operculum. Ultimately, the soft capsule passes out of the oviduct, through the mantle cavity, and along a temporary groove on the right side of the foot to the ventral pedal gland. Within this latter gland, the capsule is sculptured into a species-specific shape, hardened, and attached to a substrate.

The time an embryo spends within the egg capsule is variable (Fretter and Graham, 1962). Some gastropod embryos emerge within a week as swimming premetamorphic veliger larvae (Scheltema, 1967), so that hatching can be a relatively early developmental

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event. Other snails crawl out of capsules as postmetamorphic juveniles, after three to four months of encapsulation (Harasewych, 1978).

Three different hatching mechanisms have been described for gastropods. First, some egg capsules take up water and rupture as a result of an increased internal osmotic pressure. Capsules of *Littorina littorea* swell from an average diameter of 156 μm to 229 μm just prior to hatching, and hatching can be accelerated in dilute sea water or slowed in concentrated sea water (Davis, 1968). Second, some embryos use parts of their bodies to mechanically tear the capsule. *Lymnaea stagnalis* embryos begin normal feeding movements of the radula to rasp a hole in the capsule wall (Vaughn, 1953). Third, some gastropods escape from the capsule by chemically dissolving a pre-designated region of the capsule, termed the operculum or plug. Chemical hatching has been described for several gastropod embryos, including the oyster drill, *Urosalpinx cinerea* (Hancock, 1956), the whelks *Busycon carica* and *B. canaliculatum* (Harasewych, 1978), and the mud snail, *Ilyanassa obsoleta* (Pechenik, 1975).

Chemical hatching from other types of extraembryonic envelopes (fertilization envelope, chorion) as in sea urchins, amphibians, and fish, has been studied in detail. In these systems, the hatching enzymes are proteases that digest proteins of the envelope surrounding an embryo (see Sullivan and Bonar, 1984). However, the nature of the hatching substances released by gastropod embryos and how they function in the hatching process remain largely unknown.

In the present study, the organization of the egg capsule of *Ilyanassa obsoleta* was examined to determine how the capsule is formed and to identify the components of the capsule that the hatching substance dissolves. The *Ilyanassa* capsule is composed of four structural layers (L1–L4) that are secreted by cells of the oviduct. The capsule is covered by an outer fibrous layer (L0) deposited by cells of the ventral pedal gland. Several of these layers are located in the capsule apex and pose a barrier to hatching. The three outer layers of the capsule wall (L1, L2, and L3) and the capsule plug, a continuation of L3, are composed of protein and carbohydrate. Isolated capsule plugs contain three major glycoproteins having molecular weights of 49,000, 29,000, and 24,000 daltons. Because the hatching substance dissolves protein and carbohydrate components of the capsule plug region, it may be a protease or carbohydrase.

Materials and Methods

Biological material

Ilyanassa adults were collected from mud flats near Lewes, Delaware or in Woods Hole, Massachusetts. The snails were maintained in artificial sea water (Instant Ocean) at 10°C. To initiate reproduction, 35–40 adults were transferred to a five gallon aquarium containing aerated Instant Ocean (pH 8.0, 28‰) at 23°C, and were fed fresh clam meat regularly. Within a few days, egg capsules were produced. It was possible to observe the final stages of capsule formation (when the capsule passes from the mantle cavity to the foot) and collect capsules during this time. For the other studies, fully formed capsules were collected from the sides of the aquarium where females deposited capsules in great numbers. All capsules examined in this study were less than 24 hours old.

Electron microscopy

To prepare specimens for electron microscopy, newly collected capsules were cut open at the base and were washed repeatedly to remove embryos and capsule fluid. Capsule fragments were fixed for a minimum of 24 hours in 2.5% glutaraldehyde,

prepared in Millipore filtered sea water. Following several rinses in filtered sea water, samples prepared for transmission electron microscopy were postfixed in 1% aqueous OsO_4, dehydrated through an ethanol series, and embedded in Epon (Luft, 1961). Thin sections were cut with glass or diamond knives on a Sorvall MT-2B ultramicrotome, collected on 75 × 300 mesh copper grids (Pelco, Inc.), doubly stained with 2% aqueous uranyl acetate and 0.2% aqueous lead citrate (Venable and Coggeshall, 1965), and examined at 75 kV on a Hitachi HU-12 or at 50 kV on an RCA EMU-3H transmission electron microscope.

Capsules prepared for scanning electron microscopy were similarly fixed and washed repeatedly with distilled water, dehydrated through ethanol, and critical point dried from CO_2 (Denton DCP-1). Capsules were mounted on aluminum stubs, coated with gold-palladium (60:40), and viewed at 10 or 20 kV on an AMR 1000A scanning electron microscope. To view internal capsular structure, capsules were cut with razor blades prior to fixation or dehydrated capsules were frozen in liquid nitrogen and cryofractured with a cold razor blade before critical point drying (Humphreys *et al.,* 1974).

Histochemistry

Two approaches to determining the histochemical composition of the capsule were utilized. Capsules were fixed in 2.5% glutaraldehyde and prepared as described above for transmission electron microscopy except the postfixation in osmium was omitted. Thick (1 μm) sections of plastic embedded capsules were mounted on slides, the Epon removed in sodium methoxide (Steffens, 1978) and the sections stained, as described below. Alternatively, fresh capsules were fixed in Carnoy's fixative (Humason, 1979) and stained, then embedded in Epon and sectioned. Histochemical tests for protein (dinitrofluorobenzene), basic amino acids (fast green), aromatic amino acids (Baker's test), carbohydrates (periodic acid-Schiff's), acid mucopolysaccharides (alcian blue), metachromasia (toluidine blue), and lipid (sudan black) were performed (Pearse, 1968; Chayen *et al.,* 1973). Positive results with alcian blue that could be due to sulfate groups or carboxyl groups, were distinguished by including an increasing concentration of $MgCl_2$ in different preparations of 0.1% alcian blue (pH 2.5). Staining of sulfate groups occurs above 0.8 M $MgCl_2$ while staining of carboxyl groups occurs below 0.2 M $MgCl_2$. Both groups stain at intermediate concentrations of $MgCl_2$ (Pearse, 1968).

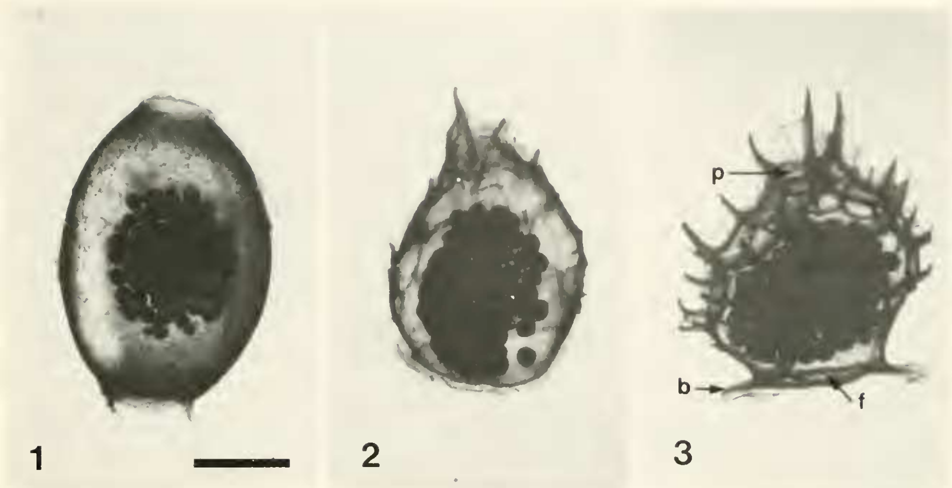
Polyacrylamide gel electrophoresis

Capsule plugs were dissected from approximately 60 newly deposited capsules, washed repeatedly in distilled water, and homogenized in 50 μl of 1% sodium dodecyl sulfate (SDS). The homogenate was added to an equal volume of 2× sample buffer (10% mercaptoethanol, 4% SDS, 20% glycerol, .002% bromophenol blue in 125 mM Tris, pH 6.8) and examined by SDS-polyacrylamide gel electrophoresis (Laemmli, 1970). Gels were stained for protein with Coomassie blue (Weber and Osborn, 1969) and for carbohydrate by the periodic acid-Schiff's stain (Fairbanks *et al.,* 1971). Molecular weight standards were co-electrophoresed with samples for determination of the molecular weights of plug proteins (Weber and Osborn, 1969).

Results

Capsule organization

The final stages of capsule formation in *Ilyanassa* are very similar to those described for the closely related snail *Nassarius reticulatus* (Fretter, 1941). A biconvex, smooth



FIGURES 1–3. Light micrographs of the final steps of capsule formation in *Ilyanassa obsoleta.* A biconvex capsule (Fig. 1) emerges from the oviduct enclosing a mass of embryos and is passed to the pedal gland in the foot. A capsule remains in the pedal gland for 15–16 minutes, but after 7–8 minutes sculpturing of the capsule is underway (Fig. 2). The plug region (p), basal disk (b), and floor of the embryo chamber (f) are easily distinguished in the mature capsule (Fig. 3). Bar = 0.5 mm.

walled capsule emerges from the mantle cavity (Fig. 1), that is transferred to the pedal gland by muscular activity of the foot. Within the pedal gland, sculpturing of the capsule surface is already underway after eight minutes (Fig. 2) and by 15–16 minutes, a hardened and fully sculptured capsule emerges from the pedal gland (Fig. 3). Mature egg capsules vary from 1–3 mm in length and 1–2 mm in width. Although neither end of a capsule can be identified as "plug end" or "base" before a capsule enters the pedal gland, the end of the capsule deepest in the pedal gland becomes the apical end containing the plug, while the basal disk protrudes from the foot and is cemented to the substratum (Fig. 3).

The egg capsule wall consists of four distinct layers, designated L1–L4 (following the scheme described for *Urosalpinx* capsules by Tamarin and Carriker, 1967; Table I). These are visible when capsules are examined by transmission electron microscopy (Fig. 4). A "typical" egg capsule is 2 mm long and 1 mm wide, has a

TABLE I

Histochemical composition of the Ilyanassa *egg capsule*

Stain	Specificity	Result
Dinitrofluorobenzene	Proteins	All layers positive
Fast Green	Basic Amino Acids	L1, L2 positive
Baker's Test	Tyrosine	L1, L3, plug positive
Periodic Acid-Schiff's	Carbohydrates	All layers positive
Alcian Blue	Acid Mucopolysaccharides	
0–0.2 *M* $MgCl_2$	COOH groups	L3 and plug positive
0.2–0.6 *M* $MgCl_2$	SO_4 and COOH groups	L3 and plug positive
0.8 *M* $MgCl_2$	SO_4 groups	All layers negative
Toluidine Blue	Metachromasia of acidic groups	L1, L3, plug positive
Sudan Black	Lipids	All layers negative

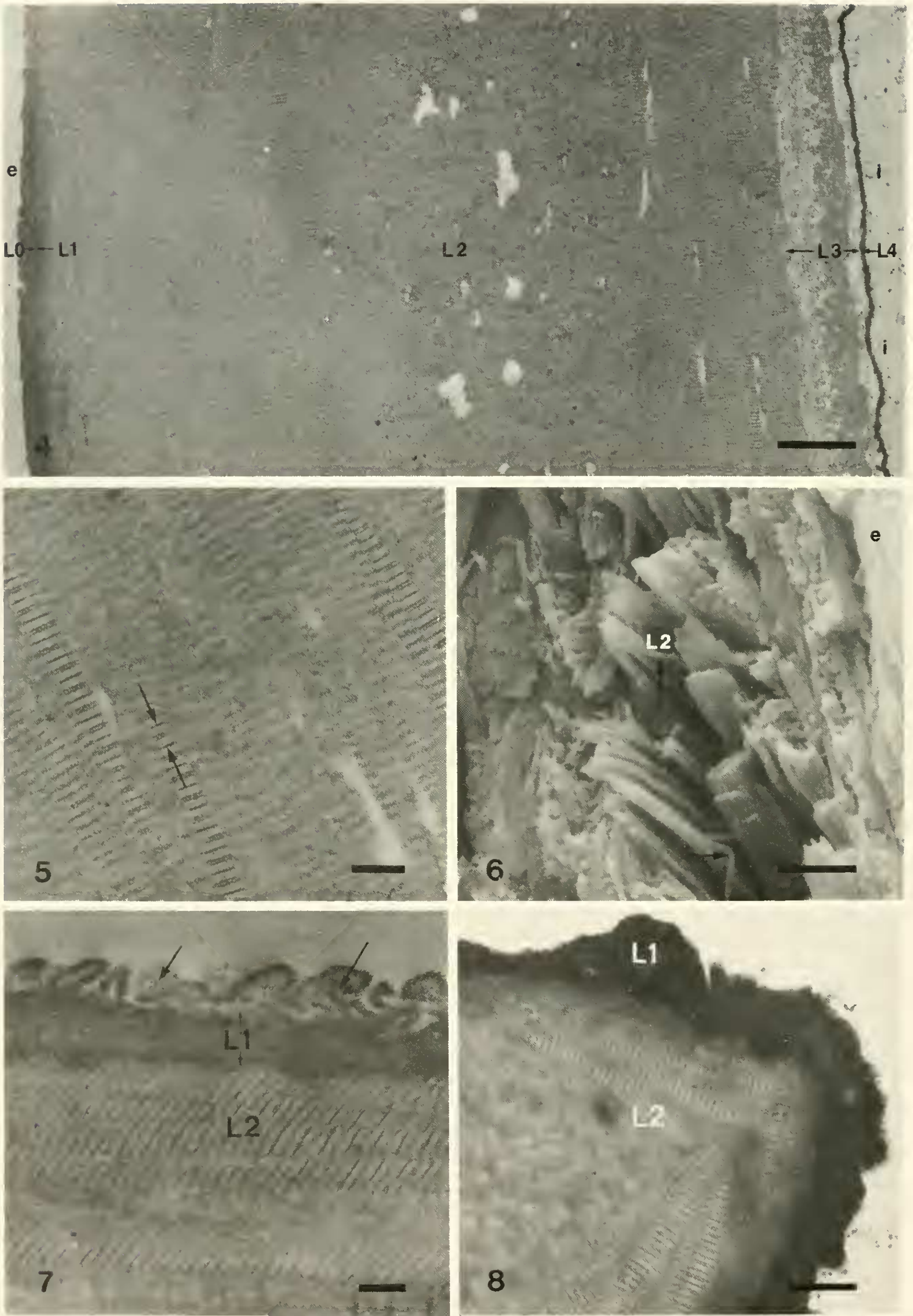


FIGURE 4. Transmission electron micrograph (TEM) of a longitudinal section of the capsule wall from a mature capsule of *Ilyanassa obsoleta.* The locations of the four layers of the capsule (L1–L4) as well as the fibrous surface layer (L0), the capsule exterior (e), and interior (i) are indicated. Bar = 1 μm.

wall 11–12 μm thick and is described in detail below. The surface of a capsule is covered by a fibrous, electron-dense layer (L0) that is not considered part of the capsule wall proper and will be discussed later. Underneath the fibrous layer is the first true wall layer (L1), which is an electron-dense layer surrounding most of the capsule. The material of this layer is quite uniform in thickness (200–250 nm) in mature capsules. Over 80% of the capsule wall (9–10 μm) consists of striated fibers located in the second layer (L2). The striations run perpendicular to the long axis of a fiber and have a periodic pattern with a repeat of 90–100 nm (Fig. 5). Single fibers are usually 60–80 nm in diameter, with the fibers organized into three bundles. Two groups of fibers are visible in longitudinal sections of the capsule, one close to L1 and the other in contact with the adjacent third layer (Fig. 4). The other bundle of fibers occupies the central region of L2 and is seen only in cross sections of a capsule. This layer of the capsule wall appears very homogeneous by scanning electron microscopy when capsules were cut with a razor blade prior to fixation (see Fig. 9). However, ethanol cryofracture of the capsule wall shows the orientation of the bundles of L2 fibers (and some individual fibers) very clearly (Fig. 6). The third layer of the wall (L3) is approximately 1.0 μm thick and consists of two groups of loosely packed 10 nm filaments on either side of a central electron-dense material (Fig. 4). Finally, the innermost portion of the wall (L4) is an electron-dense layer measuring 60 nm in thickness that completely lines the embryo chamber in all regions of the capsule. The dimensions of the layers of the wall must be regarded as approximations due to the variability of the overall size of individual capsules and to an average 5–10% shrinkage of the capsule during specimen preparation.

Capsules collected from the mantle cavity (Fig. 1) and mature capsules (Fig. 3) contain all four capsule wall layers described above, demonstrating that these layers are secreted along the oviduct. In addition, mature capsules are covered by a surface layer that is visible in electron micrographs. In thin sections, this outer layer (L0) is studded with 11–12 nm granules and usually varies from 100 to 200 nm in thickness (Fig. 7), though occasionally it reaches 500 nm in thickness. This surface layer is only present on capsules that have been sculptured in the foot and is absent from capsules collected directly from the mantle cavity (Fig. 8). In scanning electron micrographs of the surface of mature capsules L0 appears as a fibrous network that varies in thickness in different regions of the surface (Fig. 9).

Although all layers of the capsule wall described above extend into the capsule apex, their organization here is more complex. L1 and L2 occupy the outer regions of the apex (Fig. 10), as they do in the wall, but L3 expands in the central region of the apex to form most of the capsule plug (see Fig. 13). The plug is a small disk measuring 100–150 μm in thickness and 400–500 μm in diameter. The 10 nm filaments of L3 are also present in the plug but they appear more compact than they are in the wall and are arranged in a tight meshwork with filaments parallel and perpendicular to the plane of section (Fig. 11). Two other layers of the capsule contribute material

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FIGURE 5. TEM of the fibers of L2 from the *Ilyanassa obsoleta* capsule wall. The 100 nm periodicity of the striations (between the arrows) is repeated along the length of a fiber. Bar = 0.2 μm.

FIGURE 6. Scanning electron micrograph (SEM) of an oblique view of the inside of the *Ilyanassa obsoleta* egg capsule wall following ethanol cryofracture. Individual fibers (arrows) and fiber bundles are visible in several orientations. Bar = 2.0 μm.

FIGURE 7. TEM of the outer region of the mature capsule wall of *Ilyanassa obsoleta* showing the granular surface layer L0 (arrows), L1, and a portion of L2. Bar = 0.2 μm.

FIGURE 8. TEM of the egg capsule of *Ilyanassa obsoleta* wall after a capsule has emerged from the oviduct but before entering the foot (see Fig. 1). L2 is present, but L1 is less uniform in thickness than in mature capsules, and L0 is absent. Bar = 0.4 μm.

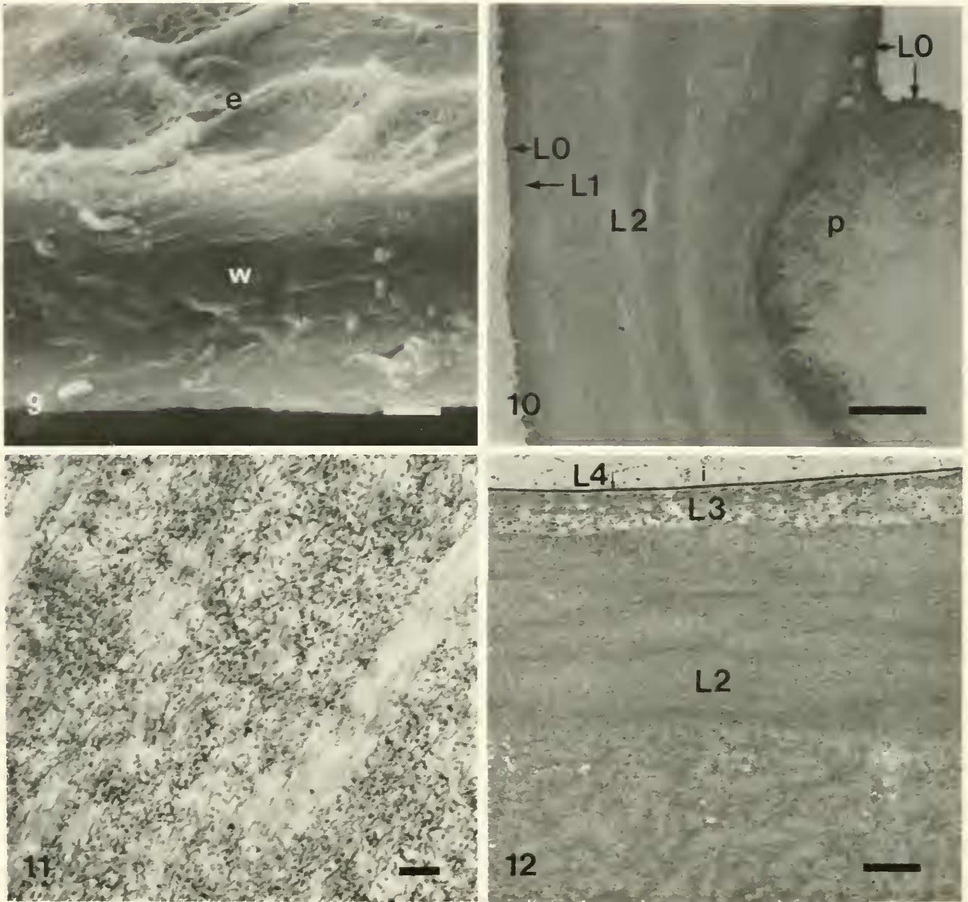

FIGURE 9. SEM of the *Ilyanassa obsoleta* egg capsule wall (w). The capsule was cut along its long axis before fixation to expose the interior of the wall. The exterior of the capsule wall (e) is covered with fibrous material. Bar = 2 μm.

FIGURE 10. TEM of the apical region of the egg capsule of *Ilyanassa obsoleta* in the region of contact between the plug (p) and the capsule wall (see asterisk in Fig. 13 for reference). L0 is visible around the outside of the capsule wall and on top of the plug. The spine adjacent to the plug contains primarily fibers of L2. Bar = 1 μm.

FIGURE 11. High magnification TEM of the capsule plug filaments from a newly deposited capsule of *Ilyanassa obsoleta.* Filaments are visible parallel and perpendicular to the plane of section. Bar = 0.1 μm.

FIGURE 12. TEM of the floor of the embryo chamber of the *Ilyanassa obsoleta* egg capsule (see "f" on Fig. 3 for reference). Starting from the interior of the capsule (i), layers L4, L3, and a portion of L2 are present. Bar = 1 μm.

to the plug. L0 is present over the surface of the capsule wall and plug in the apex (Fig. 10), while L4 is present as the innermost layer of this region.

The floor of the embryo chamber (Fig. 12) contains at least three of the structural layers of the capsule wall and like the wall, most of the floor consists of the fibers of L2. However, because capsules were collected by scraping them off the sides of the aquarium with razor blades, it is difficult to conclude whether L1 is present because L1 (along with a portion of L2) may be left behind explaining why they are not

present in micrographs of this area. Also, because the embryo chamber floor and the basal disk extending from it protrude from the pedal gland, they probably are not covered with the material of L0.

The numerous projections on the capsule surface and the basal disk (Fig. 3) contain primarily the fibers of L2 indicating that this layer is extensively redistributed during the sculpturing of the capsule that occurs in the foot. The material of L1 is also redistributed by ventral pedal gland activity. Before capsules are sculptured, L1 can vary from 100–500 nm in thickness (Fig. 8), but L1 is moulded into a more uniform layer while in the pedal gland (Fig. 7).

Histochemistry

The *Ilyanassa* egg capsule is composed of protein and carbohydrate. With the exception of sudan black, stains utilized in this study were positive for at least some layers of the capsule wall and plug. At the level of light microscopy, L1 could not be differentiated from L0, so that the results for L1 include both layers, and L4 could not be resolved well. Therefore, the histochemical results include only L1, L2, and L3.

Results of staining thick sections of the capsule (Fig. 13) and from staining of the capsule before sectioning (Table I) show that all layers of the *Ilyanassa* capsule wall contain protein, but only L1, L3, and the plug were very positive for aromatic amino

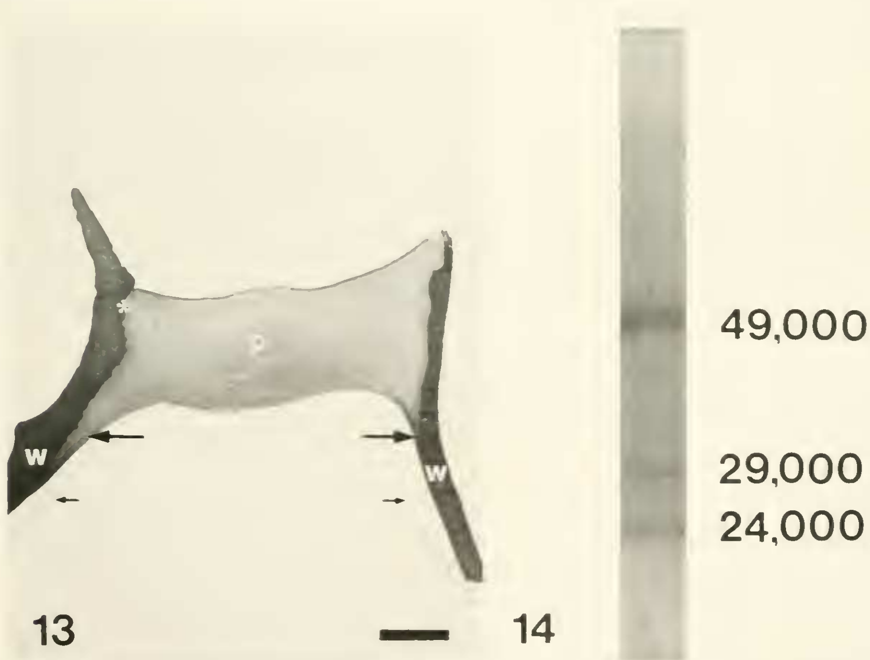

FIGURE 13. Light micrograph of a longitudinal section of the apical region of an *Ilyanassa obsoleta* capsule showing L3 of the capsule wall (large arrows) expanding to form the plug (the lighter region designated p). Some capsule fluid (small arrows) is still present internal to the capsule wall (w). The asterisk identifies the area shown at higher magnification in Figure 10. The capsule has been stained with a polychrome stain (Richardson *et al.*, 1960). Bar = 100 μm.

FIGURE 14. Analysis of the major proteins obtained from the plugs of *Ilyanassa obsoleta* egg capsules. Proteins were separated on a 12.5% polyacrylamide-SDS gel and were stained with Coomassie blue.

acids (Baker's test) and only L1 and L2 were very positive for basic amino acids (fast green). Staining of L3 and the plug with fast green was very faint. The capsule is also composed of carbohydrate. All layers of the capsule were positive with periodic acid-Schiff's stain, but only L3 and the plug stained for acid mucopolysaccharide with alcian blue. The acidic nature of these mucosubstances was due to carboxyl groups, since L3 and the plug were stained with alcian blue containing less than 0.6 M $MgCl_2$. Staining at or above 0.8 M $MgCl_2$ was very pale, demonstrating that sulfate groups were not abundant. The acidic groups in L1, L3, and the plug were organized in the proper orientation to produce a reddish-purple metachromasia with toluidine blue. Metachromasia, the shifting of the color of toluidine blue from blue to red, is produced when the acidic groups to which the dye binds are arranged closely (less than 5 Å, Pearse, 1968) so that individual dye molecules can interact. L1 and L2, being periodic acid-Schiff's positive and alcian blue negative, were composed of neutral polysaccharides.

Polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis of capsule plugs revealed three major glycoproteins with approximate molecular weights of 49,000, 29,000, and 24,000 daltons (Fig. 14). The 49,000 dalton band was the major Coomassie blue positive band while the 29,000 dalton band was the major periodic acid-Schiff's positive band. The specificity of the periodic acid-Schiff's procedure for carbohydrate moieties was verified when ovalbumin was the only molecular weight standard detected by the stain.

Discussion

The arrangement of the *Ilyanassa* capsule into several layers is similar to other egg capsules and, therefore, may be a common feature of gastropod capsule organization (Table II). However, there are differences among gastropods in the arrangement and formation of their egg capsules and minor differences in composition.

Several differences are apparent when comparing the ultrastructure of the capsule of *Ilyanassa* to that of *Urosalpinx,* the only other prosobranch gastropod capsule

Table II

Organization of the egg capsule walls of five prosobranch gastropod molluscs

Animal	Outer layer		Inner layer		Authors
Urosalpinx cinerea	L1 (30 μm)	L2 (75 μm)	L3 (2 μm)	L4 (2 μm)	Tamarin and Carriker, 1967
Nucella lapillus	Mucopoly-saccharide layer	L2 { Circular fibrous layer Long. fibrous layer Outer homogeneous layer	Inner homogeneous layer	Not detected	Bayne, 1968
Busycon carica *B. canaliculatum*	L1	L2 { Long. fibrous zone Circular fibrous zone Outer homogeneous zone	L3	L4	Harasewych, 1978
Ilyanassa obsoleta	L1 (0.2 μm)	L2 { Long. Fibers Circular Fibers Long. Fibers (9–10 μm)	L3 (1.0 μm)	L4 (60 nm)	This study

examined in detail by transmission electron microscopy (Tamarin and Carriker, 1967). The outer layer of the *Urosalpinx* capsule is a very diffuse network of striated fibers arranged circularly around the capsule. The striated pattern of these fibers has a periodicity of 53 nm, which Tamarin and Carriker (1967) termed "reminiscent of collagen." These fibers also make up the second layer and are arranged in concentric circular and longitudinal bundles. Occasionally, spaces or "vacuoles" are seen between the fibers of L2. The third layer of the *Urosalpinx* capsule is an aggregation of 5 nm filaments, while L4 contains some of the striated fibers seen in other layers and a thin inner "amorphous," electron-dense layer. In the *Ilyanassa* capsule, however, L1 is electron-dense and compact, with fibrous material (L0) present on the capsule surface external to L1. The striated fibers of the *Ilyanassa* capsule wall are restricted to L2 and are seen in all planes of section as two groups of longitudinal fibers around a central, circular band. The periodicity of the striated pattern of these fibers (90–100 nm) is very similar to the pattern seen in the capsule wall fibers of *Buccinum undatum,* which have a 100–110 nm periodicity in an alpha-helical pattern (Flower *et al.,* 1969) and are termed "keratin-like" (Rudall, 1968). The vacuoles seen between the fibers of L2 in *Urosalpinx* (Tamarin and Carriker, 1967) are occasionally seen as clear areas between the striated fibers in the walls of the *Ilyanassa* capsule (Figs. 4, 5). The size of the filaments in L3 of *Urosalpinx* and *Ilyanassa* is comparable, but in *Urosalpinx* L3 is not continuous with the capsule plug. Rather, the lower portion of the plug of the *Urosalpinx* capsule is composed of the same material found in L2 (Hancock, 1956; Tamarin and Carriker, 1967) while only the outer portion of the plug contains the filaments found in L3. A "chromotropic zone" of diffuse material, which lies around the plug separating it from the capsule walls (Tamarin and Carriker, 1967), may be functioning as a "cementum" holding the plug in place until hatching (Hancock, 1956). Finally, the thin electron-dense layer that makes up all of L4 in *Ilyanassa* capsules is only the innermost portion of a more complex L4 in *Urosalpinx.*

There is no evidence from the arrangement of the four layers of the *Ilyanassa* egg capsule wall that the embryos are inserted into an already secreted capsule as described by Fretter (1941) for the large capsules of *Nucella lapillus.* The relatively small size of the *Ilyanassa* capsule in relation to the size of the capsule gland along the oviduct (about one third the size of the gland) may allow for the sequential secretion of layers of the capsule wall around the mass of embryos that were released prior to capsule formation as occurs during the formation of the capsule around *Nassarius* eggs (Fretter, 1941). The concentric arrangement of the layers of the *Ilyanassa* capsule wall around the embryos supports the latter mechanism: L4 and L3 entirely surround the mass of embryos, while L2 and L1 are present as the outer regions of the capsule except on the plug itself.

There has been discussion whether the ventral pedal gland contributes to capsule formation (Fretter and Graham, 1962). At least four events occur while a capsule is maturing in the ventral pedal gland: sculpturing of the capsule surface by a redistribution of layers L1 and L2; hardening of the capsule wall, possibly by chemical cross-linking (Price and Hunt, 1973, 1976); fibrous material is added onto the capsule surface; and the capsule is attached by its basal disk to some substrate. Our study shows that the four structural layers of the *Ilyanassa* egg capsule wall identified by transmission electron microscopy originate in the oviduct so that the ventral pedal gland does not secrete any of the structural layers of the capsule wall in *Ilyanassa.* However, the layers are modified while a capsule is in the pedal gland. The chemical reactions occurring during hardening of the capsule wall may explain the different electron densities of L1 observed before and after capsules have passed through the gland (compare Figs. 7 and 8).

TABLE III

Comparison of the histochemical composition of the capsules of Nucella lapillus *(Bayne, 1968) and* Ilyanassa obsoleta

	Ilyanassa			Nucella		
Compound	L1	L2	L3	L1	L2	L3
Protein	+	+	+	+	+	+
Basic Amino Acids	+	+	very pale	+	+	+
Tryosine	+	−	+	+	+	0
Carbohydrate	+	+	+	+	+	+
Acid Mucopolysaccharide	−	−	+	+	−	+
Metachromasia	+	−	+	+	−	+
Lipids	−	−	−	−	−	−

+ = Positive.
− = Negative.
0 = Not Tested.

The capsule of only one other prosobranch gastropod has been examined by histochemistry. Bayne (1968) found that the *Nucella* egg capsule is also composed of carbohydrate and protein, with no evidence of lipid being present (Table III). Our results for the *Ilyanassa* capsule are in excellent agreement with those for *Nucella* indicating the capsules of these two prosobranchs have a similar composition as well as a similar organization. The only differences in histochemical composition between the two capsules were the detection of acid mucopolysaccharides in L1 of *Nucella* and minor differences in amino acid composition (Bayne, 1968).

As discussed earlier, many embryos release enzymes in order to hatch from egg envelopes. It is not known whether the hatching substance released by *Ilyanassa* embryos to dissolve the capsule plug is also an enzyme. However, if it is, it must be able to dissolve L4 and L0 as well as the protein and/or carbohydrate components of L3. Protease activity is released from *Ilyanassa* embryos at the time of hatching (Sullivan and Bonar, 1984) and our histochemical tests have identified amino acid groups cleaved by chymotrypsin (aromatic amino acids) and trace amounts of those cleaved by trypsin (basic amino acids). The dual carbohydrate and protein composition of gastropod egg capsules has been measured more directly by chemical analysis (Hunt, 1966). The capsules of *Buccinum* contain 78% protein and only 8% carbohydrate by weight. Our results from gel electrophoresis and histochemistry demonstrate that the plug region of *Ilyanassa* capsules dissolved during hatching also has a dual composition. Our plug samples do contain small portions of L0 and L4, but because most of the plug region is L3, we suspect that all three glycoproteins identified are from the latter layer.

We are currently examining the ability of several proteases to mimic the action of the hatching substance and we are trying to determine if the hatching substance degrades any of the three plug glycoproteins in order to elucidate a mechanism of action of the *Ilyanassa* hatching substance.

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